

Abnormal action potentials associated with the *Shaker* complex locus of *Drosophila*

(behavior/neurogenetics/potassium channel/membrane physiology/gene complex)

MARK A. TANOUYE, ALBERTO FERRUS*, AND SHINOBU C. FUJITA

Division of Biology, California Institute of Technology, Pasadena, California 91125

Contributed by Seymour Benzer, July 20, 1981

ABSTRACT Intracellular recordings of action potentials were made from the cervical giant axon in *Shaker* (*Sh*) mutants and normal *Drosophila*. The mutants showed abnormally long delays in repolarization. The defect is not due to abnormal Ca^{2+} channels, because it persists in the presence of Co^{2+} , a Ca^{2+} -channel blocker. On the other hand, the K^{+} -channel blocker 4-aminopyridine causes a similar effect in normal animals, suggesting that the *Sh* mutant may have abnormal K^{+} conductance. Gene-dosage analysis of *Sh* shows that the defect is not due to underproduction of an otherwise normal molecule; it may be due to an abnormal molecule produced by the mutated gene. Gel electrophoresis failed to detect an abnormal protein, suggesting that, if *Sh* codes for a nervous system protein, it is rare. Genetic analysis of the *Sh* locus indicates three regions. Mutations or chromosome breaks in the two flanking regions cause *Sh* mutant physiology; the central region shows a "haplolethal effect"—i.e., heterozygous females are lethal.

Mutations that alter neuronal physiology can be used to probe basic mechanisms underlying nervous system function. Flies mutant for the *Shaker* (*Sh*) locus in *Drosophila melanogaster* were detected originally because they shake their legs vigorously under anesthesia (1–3). Behavioral abnormalities (wing scissoring, abdominal spasms, antennal twitching) are also observable in unanesthetized *Sh* adults. Jan *et al.* (4) found that *Sh* mutant larvae show abnormally prolonged transmitter release at the neuromuscular junction and suggested that this might be due to defective K^{+} channels in the nerve terminal membrane. In this report, we describe abnormalities in the nerve action potential of *Sh* mutants by using intracellular recordings and present a detailed genetic analysis of the *Sh* locus.

MATERIALS AND METHODS

Mutants and Chromosome Rearrangements. The *Sh* alleles used in this analysis, *Sh*⁵, *Sh*^{E62}, *Sh*¹⁰², *Sh*^{rK0120}, and *Sh*^{KS133}, were all ethyl methanesulfonate-induced mutations. All showed normal larval salivary chromosomes in the region 16F, and none modified the recombination frequency between the markers *forked* (*f*), *fluff* (*ff*), and *outstretched-small eyes* (*os*) indicating that they may be point mutations. To analyze the *Sh* region, the following x-ray-induced chromosomal translocations were used: *T(X;Y)* V7=16F5-8 (translocation number V7 from the X chromosome, broken in salivary band region 16F5 to 16F8 to the Y chromosome), *T(X;Y)* W32=16F3-6, *T(X;Y)* B55=16F1-4 and *T(X;3)* *Sh*^{LC}=16F1-2;80. The deficiency *Df(X;3)* *JC153* and duplication *Dp(X;3)* *JC153* were obtained from the insertional translocation *T(X;3)* *JC153*=16E2-4; 17A-B;99D. In this report, the proximal element (between the breakpoint and the centro-

mere of the X) of a *T(X;Y)* will be identified by the number of the translocation with a superscript P. Superscript D indicates the distal element. For scoring, the proximal elements of all the *T(X;Y)*s are marked by *y*⁺, and the distal are marked with *B*^S. The remaining chromosomes and mutants used are described by Lindsley and Grell (5).

For gene-dosage analysis, aneuploid flies were constructed by using either the proximal element (*X*^P) of *T(X;4)* *B*^S or the duplication, *Dp(X;3)* *JC153*. These two elements contain the entire normal *Sh* region (*Sh*⁺) and other genes (5). Both aneuploidies yielded similar results.

Physiology. Adult flies (4–6 days old) were mounted ventral side up in soft wax so that the dorsal surface, including spiracles, was in contact with circulating air while the ventral surface was exposed to saline (6). The cervical connective, containing the axon of the cervical giant fiber (CGF) neuron, was exposed by removing the proplepisternum. The composition of the normal saline was that of Jan and Jan (7). The CGF was stimulated electrically in the brain by using a pair of insulated tungsten electrodes. Intracellular recordings of CGF action potentials were made using 3 M KCl-filled glass micropipettes of resistance 40–60 MΩ. CGF motor outputs (not shown) were monitored to identify physiologically CGF recording sites (6, 8). Membrane resting potentials varied from –60 to –80 mV for both normal and mutant CGF axons. CGF anatomy has been described (8).

Electrophoresis. Brains were dissected from flies freeze-dried in acetone at –20°C. This procedure inhibits proteolysis and allows clean dissection of many organs (9). Brains were homogenized (typically, 25 brains per 50 μl) in lysis buffer (10)/0.5% NaDodSO₄. After centrifugation at 2000 × *g* for 5 min, the supernatant was run at 10 brains per gel. Twelve gels were run in parallel. For neutral and acidic proteins (pI <8), the electrophoresis method of O'Farrell (10) was used. For more basic proteins (pI >6), NaDodSO₄ was omitted and electrophoresis was according to O'Farrell *et al.* (11). Gels were stained with silver (12, 13). Most samples were analyzed in duplicate.

RESULTS

Abnormal Repolarization in *Sh* Action Potentials. Intracellular microelectrode recordings of CGF action potentials in normal *Drosophila* are similar in waveform and time course to those in the squid giant axon (see Fig. 1), except that the falling phase in *Drosophila* does not undershoot the resting potential. CGF pharmacology is similar to other species (see ref. 14, for example). The action potential is blocked by the Na⁺-channel blocker tetrodotoxin (3 μM), suggesting that it involves an inward Na⁺ current. The action potential is also sensitive to the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: *Sh*, *Shaker*; CGF, cervical giant fiber.

* Present address: Centro de Biología Molecular, Universidad Autónoma, Madrid-34, Spain.

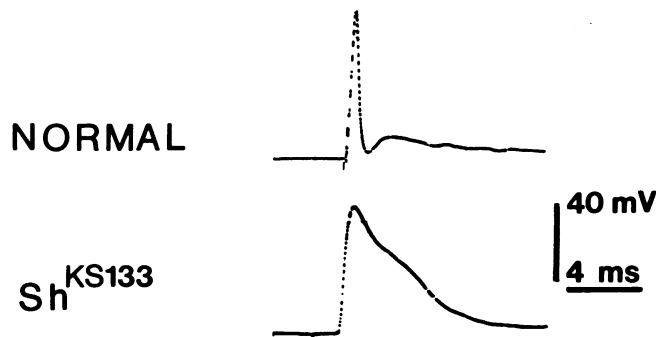


FIG. 1. Typical action potentials from normal and Sh^{KS133} mutant flies. An intracellular microelectrode records the action potential from the CGF evoked by a 0.1-ms stimulus to the brain. Sh^{KS133} shows a delay in repolarization.

K^+ -channel blocker, 4-aminopyridine, indicating an outward K^+ current (see Fig. 4). Following the spike is a small depolarizing afterpotential (see Fig. 3) that is eliminated by Co^{2+} , a Ca^{2+} -channel blocker. This Ca^{2+} component may account for the lack of undershoot in the CGF action potential.

Fig. 1 shows an action potential for the mutant Sh^{KS133} . A delay in repolarization prolongs the time course. The duration of the action potential was defined by its full width at half maximum. The normal duration was 0.40 ± 0.06 ms (results are mean \pm SD; $n = 10$). Sh^{KS133} action potentials were longer (5.2 ± 1.0 ms). These experiments used brain stimulation. This difference between normal and mutant appears to be of axonal rather than synaptic origin because it was also observed when action potentials were induced directly by injecting current through the intracellular microelectrode, or when synaptic transmission was blocked by Co^{2+} (see Fig. 3).

Abnormal action potentials also are seen in other mutant alleles of Sh (Fig. 2). Sh^{102} , for example, is similar in waveform and time course to Sh^{KS133} . The action potentials are not, however, identical in every Sh allele. In Sh^5 , Sh^{E62} , and Sh^{rK0120} , the action potentials begin to repolarize more quickly than for Sh^{KS133} , but this initial repolarization is incomplete and followed by multiple spikes. Multiple spikes following a single stimulus are seen in the action potentials of all Sh mutant alleles, although less frequently in Sh^{KS133} and Sh^{102} . They vary in number and amplitude for repeated stimuli delivered to a single animal and among animals of the same genotype. Multiple spikes are considered to be a secondary consequence of the re-

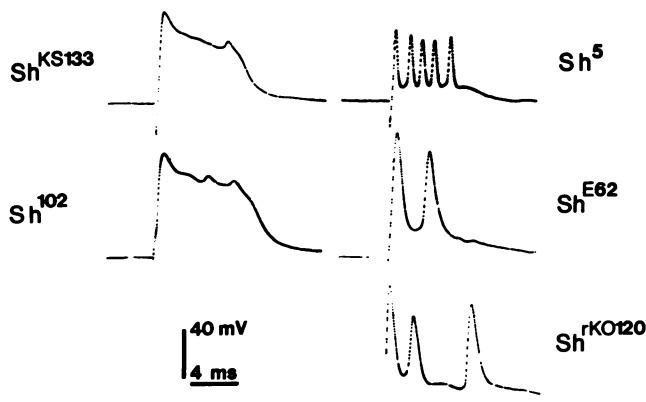


FIG. 2. Action potentials for five Sh alleles. Sh^{KS133} and Sh^{102} show long delayed repolarization. In Sh^5 , Sh^{E62} , and Sh^{rK0120} , incomplete initial repolarization is accompanied by multiple spikes. The waveforms shown are typical of each allele.

polarization defect because of their variability, sensitivity to Co^{2+} block (see below), and because they accompany delayed repolarization in other preparations (15, 16). Multiple spikes have also been reported for Sh larvae (17, 18).

Sh is Not Explained by a Ca^{2+} -Channel Defect. A delay in repolarization could be produced by abnormally large inward Ca^{2+} currents acting to keep the nerve axon depolarized. This possibility may be examined by using divalent cations such as Co^{2+} to block Ca^{2+} channels (14), in which case Sh action potentials should become similar to those in normal animals. The effect of (10 mM) Co^{2+} on normal and Sh^{KS133} action potentials is shown in Fig. 3. This dose of Co^{2+} was sufficient to block synaptic transmission, usually within 1 to 2 min, as monitored by CGF output pathways (not shown). Action-potential waveforms did not change after this time. Higher concentrations of Co^{2+} (20 mM) produced similar results in a shorter time, usually within 30 sec. In the normal axon, Co^{2+} eliminates the small depolarizing afterpotential and broadens the spike slightly. In Sh^{KS133} , there is some smoothing effect on the later part of the trace. Co^{2+} also suppresses all multiple spikes, suggesting that a Ca^{2+} component is necessary for their occurrence.

In the Co^{2+} -blocked state, the delayed repolarization defect persists. Similar results are seen in other Sh alleles (Sh^5 , Sh^{102}), indicating that the Sh defect is not due to an abnormal Ca^{2+} current.

K^+ -Channel Blockers Cause Normal Axons To Have Sh -Like Action Potentials. The Sh defect might be caused by an abnormal K^+ conductance that fails to repolarize the nerve properly after an action potential. If so, drugs that block K^+ channels, such as 4-aminopyridine, might give rise to similar action potentials when applied to normal animals. Indeed, that is so (Fig. 4, cf. Fig. 1). When 4-aminopyridine is applied to Sh^{KS133} animals, little change is seen in the waveform. These results confirm, on the intracellular level, those of Jan *et al.* (4) for the larval neuromuscular junction, strengthening the hypothesis that Sh mutants have defective K^+ channels.

Evidence that Sh Mutants Produce An Altered Gene Product. The Sh repolarization defect could be due to underproduction of a molecule—e.g., a component of a K^+ channel in the membrane. Alternatively, it could be due to an abnormal molecule produced by the mutated gene. To test this, a gene-dosage analysis was done based on the observation that, generally in *Drosophila*, the amount of gene product is proportional to the number of copies of a gene (19). If there is underproduction, then normal behavior and physiology should be restored by

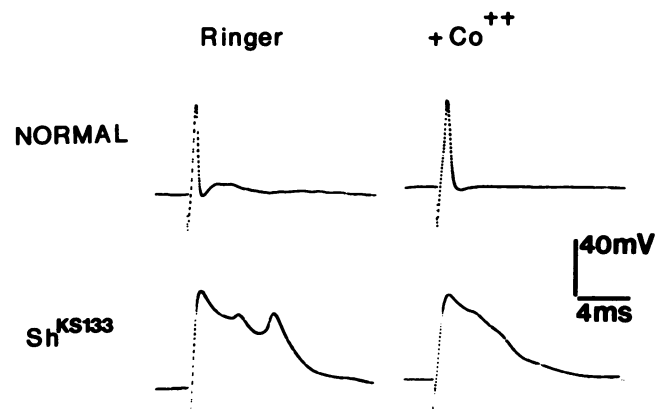


FIG. 3. Effect of Co^{2+} . Normal and Sh^{KS133} action potentials in normal Ringer solution (Left) and after addition of $CoCl_2$ to 10 mM (Right). In the normal fly, the after potential is eliminated. The spike is only slightly broadened. For Sh^{KS133} , the long delay in repolarization persists in the presence of Co^{2+} .



FIG. 4. Effect of 4-aminopyridine (4AP) on action potential of normal fly. Addition of 4-aminopyridine to 6 mM delays repolarization, producing a waveform similar to that seen in certain *Sh* mutants.

creating an animal that has a normal complement of the *Sh*⁺ gene in addition to the mutant *Sh* gene. If the *Sh* mutation encodes an altered gene product, the defect should still be present, albeit reduced.

In *Drosophila*, the activity of genes on the X chromosome is dosage compensated (20). That is, if activity in the male (with a single X chromosome) is defined as 1.0, the corresponding activity for each of the two female X chromosomes is 0.5. When a fly contains one or more additional small segments of the X chromosome (aneuploidy) the activity of these segments is determined by the sex of the fly. Fig. 5 shows, in addition to normal and mutant flies, various genetic combinations obtained among the progeny of the crosses ($\text{♀ } FM7aB^+/T(X;4)B^S \times \text{♂ } Sh^{KS133}$) or [$\text{♀ } Sh^{KS133}/FM7aB^+/X^P(T(X;4)B^S) \times \text{♂ } Sh^{KS133}$]. The addition of normal *Sh*⁺ genes to *Sh*^{KS133} appeared to reduce the severity of leg shaking under anesthesia in a progressive manner, although all these animals did continue to shake. *Sh*⁺ genes greatly improved the *Sh*^{KS133} action potential. Rather than the delayed repolarization characteristic of *Sh*^{KS133} homozygotes, the action potential defect in animals containing various proportions of *Sh*⁺ was reduced to a rapid, albeit partial, repolarization with consequent double spiking. Even in individuals having a full complement of the normal gene, the presence of the mutant gene brought about mutant behavioral and physiological phenotypes, including leg shaking and incomplete repolarization. Similar gene-dosage experiments using two other alleles, *Sh*^S and *Sh*^{KO120}, yielded comparable behavioral results but were not studied physiologically. We also constructed individuals having *Sh*^{KS133} and an excess of the normal gene [genotype: *Sh*^{KS133}/*X*^P(*T(X;4)B*^S); *Dp(X;3) JC153/+*]. These, too, showed slight abnormality in leg shaking and action potential. Flies having extra doses of the *Sh*⁺ gene but no copy of the mutant gene were also tested and all had normal behavior and normal CGF action potentials. All these observations are consistent with the interpretation that the mutant *Sh* gene encodes an abnormally functioning gene product rather than fails to produce a normal one.

Attempt to Identify a *Sh* Gene Product. Two-dimensional gel electrophoresis was done on brain proteins of the *Sh* alleles *Sh*^S, *Sh*¹⁰², *Sh*^{KO120}, *Sh*^{KS133}, deletion *B55*^D/*W32*^P, duplication *W32*^D/*B55*^P, and appropriate controls. To minimize the effects of genetic background differences, the flies used were obtained from single-pair backcrosses designed to yield mutant and normal populations having comparable autosomal backgrounds. Particular attention was given to comparison of flies carrying a small duplication of *Sh*⁺ with those having the corresponding deficiency. Approximately 1000 proteins (*M_r* > 20,000) were visible at a sensitivity of ≈ 0.1 ng for typical proteins. Neither qualitative nor quantitative differences were found that could be ascribed to the *Sh* locus. This suggests that, if the *Sh*⁺ gene product is a nervous system protein, its level is below the sensitivity of these experiments or it has unusual solubility or staining properties.

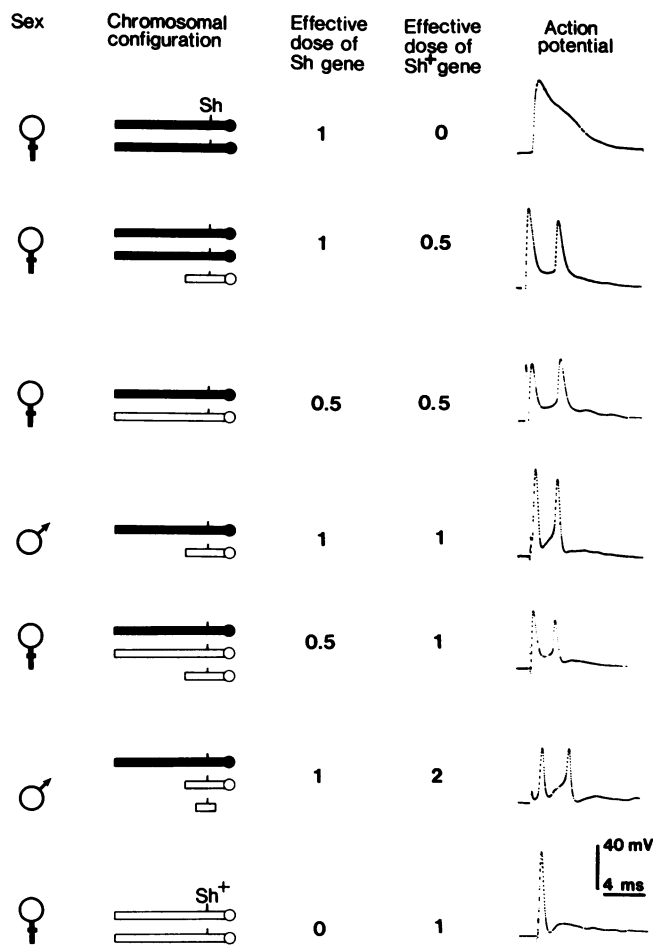


FIG. 5. Gene-dosage analysis of *Sh*^{KS133}. Action potentials from individuals carrying various combinations of mutant *Sh* (●) and normal *Sh*⁺ (○) genes. Numbers indicate the theoretical amounts of each gene product, taking into account the dosage compensation that causes X-chromosome genes to be expressed at half level when two X chromosomes are present (in females) and at full level when there is only one X chromosome (in males). At least five action potentials were recorded from each genotype. Results shown are typical for the genotypes *Sh/Sh* female (10 animals), *Sh/Sh/+* female (1 animal), *Sh/+* female (3 animals), *Sh/+* male (2 animals), *Sh/+* female (1 animal), *Sh/+* male (1 animal), and *+/+* female (10 animals). The addition of even one dose of the normal *Sh*⁺ gene modifies the *Sh*^{KS133} action potential such that the long-delayed repolarization is eliminated. Nevertheless, initial repolarization remains incomplete, which is the essential feature of the *Sh* phenotype. The extra spikes may be secondary effects. Note that, even in those genotypes in which the *Sh*⁺ gene product should be present at normal or greater than normal levels, the action potentials are not completely normal.

***Sh* is a Gene Complex.** *Sh* mutations occur in a small region that includes several bands within the division 16F on the salivary X chromosome. Four independently derived translocations having chromosome breaks at different points in the 16F region all show leg shaking and abnormal CGF action potentials (Fig. 6). The locations of these breaks are shown in Fig. 7 [genotypes: *T(X;3) Sh*^{LC}, *T(X;Y) B55*, *T(X;Y) W32*, and *T(X;Y) V7*]. The *Sh* phenotypes associated with the breaks indicate that all the chromosomes are probably broken in *Sh*. Nine other strains containing breakpoints in the intervals 16B–16E and 17A1–17A12 did not shake. The breakpoint of *T(X;3) JC153* lies just outside *Sh* because flies carrying it lack *Sh* phenotypes.

The left portion, between the breakpoints of *T(X;3) JC153* and *T(X;Y) W32*, contains the breakpoints of *T(X;3) Sh*^{LC} and

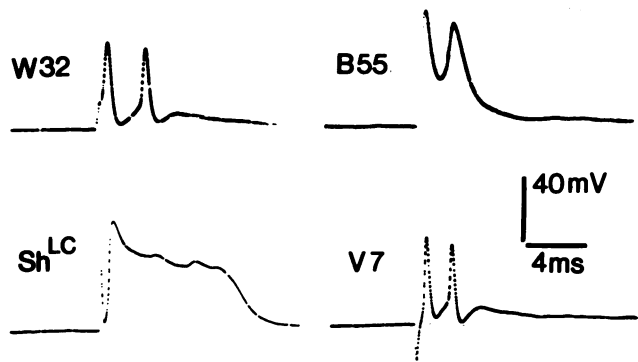


FIG. 6. Action potentials from flies that have translocations breaking at different points in the *Sh* region (break and shake). The rearrangements *T(X;Y)* W32, *T(X;Y)* B55, *T(X;Y)* V7, and *T(X;3)* *Sh*^{LC} all show abnormal action potentials. The breakpoints are shown in Fig. 7.

T(X;Y) B55. Because action potentials in *Sh*^{KS133} homozygotes are distinct from those in *Sh*^{KS133/+} flies (cf. Fig. 5), this allele was mapped to the portion of the left region between *T(X;3)* *JC153* and *T(X;Y)* B55.

The right boundary of *Sh* must be to the left of the gene *os* (Fig. 7), which does not show the *Sh* phenotype. The right-most breakpoint that causes leg shaking and CGF action potential abnormalities is *T(X;Y)* V7 (Figs. 6 and 7). To test whether this defect could be due to a second distant mutation site, recombinants were made in which the distal X chromosome of this translocation was replaced with normal X chromosome material to within 0.5 map units of the breakpoint. All recombinants still showed leg shaking. Thus far, no mutant alleles have been mapped to the right portion of *Sh*.

The central portion of *Sh* is of special interest. It is located between the two chromosomal breakpoints, *T(X;Y)* W32 and *T(X;Y)* V7 (Fig. 7). It is unusual in that a heterozygous deficiency of it (genotype: *V7^P/W32^D/FM7a*) is lethal; it is a "haplolethal." Animals die in the embryonic stage, even though they carry one normal copy of the entire *Sh*⁺ complex. Deficiency animals can be rescued by further addition of a small duplication [e.g., *Dp(X;3)* *JC153*]. The leg shaking of one viable allele *Sh*^{rK0120} is greatly reduced in heterozygotes. This allele maps to the central region; a similar reduction occurs in *Sh*^{rK0120}/*W32^P* or *Sh*^{rK0120}/*V7^D* flies. Flies having a heterozygous deficiency for the left region [genotype: *Df(X)* *JC153/FM7a/W32^P*] are viable and have abnormal action potentials and leg shaking. Flies having a heterozygous deficiency for the right region [genotype: *Df(X)* *JC153/V7^D/FM7a*] are viable and have leg shaking (action potentials have not been tested).

Thus, *Sh* seems to be a complex genetic locus organized into three different regions—two flanking regions in which mutations or chromosome breaks give rise to the *Sh* mutant phenotype and a central region that behaves as a haplolethal.

Our analysis of *Sh* depends, to a large extent, on *Sh* phenotypes associated with the breakpoints of X-Y chromosomal translocations. Because the translocation of part of the X chromosome into the Y brings the break into contact with heterochromatin, *Sh* phenotypes could conceivably arise from the influence of the heterochromatin (position-effect variegation) rather than from a break in the *Sh* region itself. This was tested for by adding an extra Y chromosome and also by culturing at high temperature procedures that are known to suppress variegation in *Drosophila* (21). Neither condition affected the *Sh* phenotype.

We have begun to saturate the *Sh* region for lethal mutations.

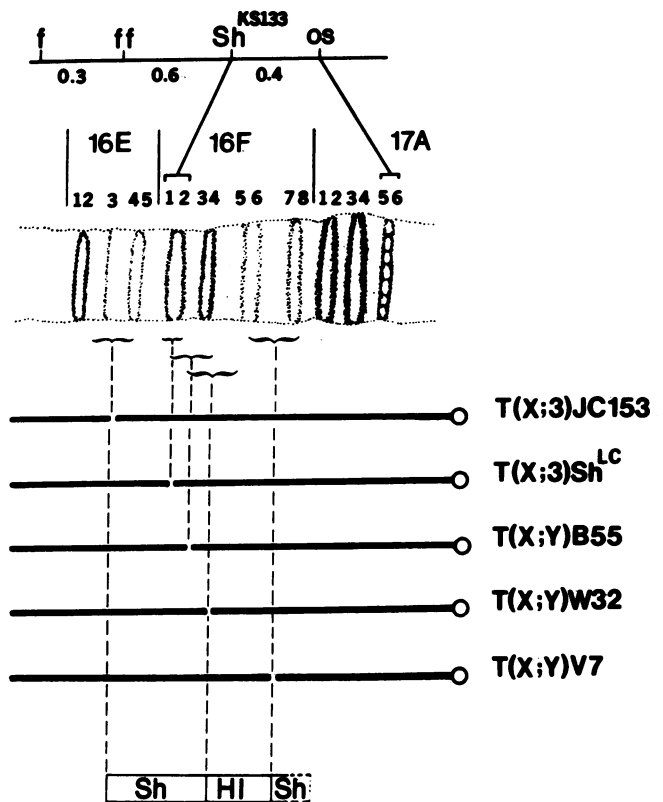


FIG. 7. Genetic organization of the *Sh* complex. *Sh*^{KS133} maps by recombination between the genes *ff* and *os* on the X chromosome. A drawing of the salivary chromosome for this region is shown. The six chromosomal rearrangements used to localize *Sh* are indicated by open circles representing the centromere (proximal end). The cytogenetic locations of their breakpoints are shown with brackets indicating the degree of uncertainty. Note that the translocated chromosomes also contain other breakpoints not associated with *Sh* [e.g., *T(X;3)* *JC153* is also broken in 17A-B and 99D] but for clarity, these other breaks are not shown. The *Sh* region is organized into three major parts. The breakpoint of *T(X;3)* *JC153* marks the left flank. The breakpoint of *T(X;Y)* W32 marks the separation between the left and central regions of *Sh*. The breakpoint of *T(X;Y)* V7 marks the separation between the central and right regions. The right limit of *Sh* is not known but must be to the left of *os*, which does not show *Sh* phenotype and has been cytogenetically mapped to the doublet 17A5-6 (26).

Thus far, nine alleles belonging to five complementation groups have been identified.

DISCUSSION

Based on their analysis of prolonged transmitter release at the neuromuscular junctions of *Sh* larvae, Jan et al. (4) suggested the possibility of a K⁺-channel defect. The present analysis used intracellular microelectrodes to show definitively that *Sh* adults have action potentials with delayed repolarization. Although various *Sh* mutants show different waveforms, repolarization defects are common to all.

Several observations in both the larval and the adult preparations suggest a defect in K⁺ conductance. Jan et al. (4) showed that abnormal transmitter release could still be evoked electrotonically at *Sh* larval neuromuscular junctions, even when all regenerative Na⁺-channel activity was blocked. That indicated that the defect is not due to delayed Na⁺-channel inactivation. The present experiments showed that *Sh* mutant physiology is probably not due to a Ca²⁺-channel defect; the abnormality is still present when Ca²⁺ channels are blocked with Co²⁺. Finally, in both larval and CGF preparations, the *Sh* abnormality

is mimicked in normal animals by the application of K^+ -channel blocking agents. Taken together, these observations suggest that the *Sh* abnormality is due to a defect in K^+ conductance that prevents proper repolarization of the membrane.

Gene-dosage experiments suggest that *Sh* mutations cause the production of a modified gene product rather than the reduction of a normal one. An interpretation of these findings is that *Sh* mutations encode abnormal K^+ channels that compete with normal K^+ channels for a limited number of membrane sites, thus interfering with normal function. We were unable to identify the gene product by electrophoretic methods. At the sensitivity of our technique, however, a brain protein present at a level <0.01 ng per brain would not have been detected. The amount of Na^+ -channel protein in a *Drosophila* head can be estimated at 0.01 ng (22, 23), but no data are available for the K^+ channel.

Association of *Sh* phenotypes with chromosome breakpoints within the 16F salivary chromosome region indicates that the first two pairs of bands are definitely part of the *Sh* complex. Within the uncertainties of cytogenetic determination, it is possible that the last band in 16E and the third pair of bands in 16F are also involved. From the total thickness of these bands and from preliminary data on recombination frequencies between point mutants in the region (≈ 0.1 map units), an estimate of the DNA contained in *Sh* would be of the order of 100 kilobases (24). The *Sh* gene appears to contain three major areas: two flanking regions in which chromosome breaks or mutations result in *Sh* phenotypes and a central region that, when deleted, results in haplolethality. Haplolethals are extremely rare; only one other is known in the *Drosophila* genome (25).

The complexity of the *Sh* region raises the possibility that it might code for different subunits of a channel or functionally related channels. Distinct K^+ currents have been described in a number of organisms, most notably molluscs (14) and it is becoming apparent that the membrane-excitability properties that distinguish neuron types are largely determined by differences in K^+ currents. Combinations of these channels in the membrane may determine whether a cell is, for example, a pace-making neuron, a bursting neuron, or one that fires with a single action potential. *Sh* mutants may contribute to our understanding of how excitability differences are organized within the nervous system by revealing how distributions of K^+ channels are controlled at the level of the genome.

We are grateful to S. Benzer for continual encouragement and support. We thank L. Kauvar, J. Reinitz, and S. Shotwell for discussion. Special thanks go to I. Duncan for cytogenetic determinations. T. Homyk, G. Lefevre, and J. Merriam kindly provided several stocks. This research was supported by National Science Foundation Grant PCM

7911771 to S. Benzer and the Pew Memorial Trust. M.A.T. was supported by a Muscular Dystrophy Association fellowship and National Institutes of Health Training Grant GM 7401-02. A.F. was supported by National Institutes of Health Fellowship FO5 TWO2477 and a Gossney Foundation fellowship.

1. Catsch, A. (1944) *Z. Ind. Abst. Vererb.* **72**, 119–126.
2. Kaplan, W. D. & Trout, W. E., III (1969) *Genetics* **61**, 399–409.
3. Trout, W. E., III & Kaplan, W. D. (1973) *J. Neurobiol.* **4**, 495–512.
4. Jan, Y. N., Jan, L. Y. & Dennis, M. J. (1977) *Proc. R. Soc. London Ser. B* **198**, 87–108.
5. Lindsley, D. L. & Grell, E. H. (1968) *Carnegie Inst. Washington Publ.* **627**.
6. Tanouye, M. A. & Wyman, R. J. (1980) *J. Neurophysiol.* **44**, 405–421.
7. Jan, L. Y. & Jan, Y. N. (1976) *J. Physiol. London* **262**, 189–214.
8. Koto, M., Tanouye, M. A., Ferrus, A., Thomas, J. B. & Wyman, R. J. (1981) *Brain Res.*, in press.
9. Fujita, S. C. & Hotta, Y. (1979) *Prot. Nucleic Acid Enz.* **24**, 1336–1343 (Japanese).
10. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021.
11. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* **12**, 1133–1142.
12. Switzer, B. C., Merrill, C. R. & Shifrin, S. (1979) *Anal. Biochem.* **98**, 231–237.
13. Oakley, B. R., Kirsch, D. R. & Morris, N. R. (1980) *Anal. Biochem.* **105**, 361–363.
14. Adams, D. J., Smith, S. J. & Thompson, S. H. (1980) *Annu. Rev. Neurosci.* **3**, 141–167.
15. Ramon, F. & Moore, J. W. (1979) *J. Gen. Physiol.* **73**, 595–603.
16. Kaars, C. & Faber, D. S. (1981) *Science* **212**, 1063–1064.
17. Ganetzky, B. & Wu, C.-F. (1980) *Neurosci. Abstr.* **6**, 569 (abstr.).
18. Jan, Y. N. & Jan, L. Y. (1979) in *Insect Neurobiology and Pesticide Action*, ed. Rickett, F. E. (Soc. Chem. Industry, London), 161–168.
19. MacIntyre, R. J. & O'Brien, S. J. (1976) *Annu. Rev. Genet.* **10**, 243–253.
20. Stewart, B. & Merriam, J. (1980) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Wright, T. (Academic, New York), Vol. 2, pp. 107–140.
21. Spofford, J. B. (1976) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Novitski, E. (Academic, New York), Vol. 1, pp. 955–1009.
22. Kauvar, L. M. (1979) *Caltech Biol. Annu. Rep.* **154** (abstr.).
23. Agnew, W. S., Moore, A. C., Levinson, S. R. & Raftery, M. A. (1980) *Biochem. Biophys. Res. Commun.* **92**, 860–866.
24. Chovnick, A., Gelbart, W. & McCarron, M. (1977) *Cell* **11**, 1–10.
25. Lindsley, D. L., Sandler, L., Baker, B. S., Carpenter, A. T. C., Denell, R. E., Hall, J. C., Jacobs, P. A., Miklos, G. L. G., Davis, B. K., Gethmann, R. C., Hardy, R. W., Hessler, A., Miller, S. M., Nozawa, H., Parry, D. M. & Gould-Somero, M. (1972) *Genetics* **71**, 157–184.
26. Lefevre, G., Jr. (1976) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Novitski, E. (Academic, New York), Vol. 1, 31–66.